Effects of Ultrasonic and Microwave Pretreatment on Lipid Extraction of Microalgae and Methane Production from the Residual Extracted Biomass

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Abstract

The extraction of lipids from microalgae cells of *Botryococcus braunii* and *Chlorella vulgaris* after ultrasonic and microwave pretreatment was evaluated. Cell disruption increased the lipid extraction efficiency, and microwave pretreatment was more effective compared with ultrasonic pretreatment. The maximum lipid yield from *B. braunii* was 56.42% using microwave radiation and 39.61% for ultrasonication, while from *C. vulgaris*, it was respectively 41.31% and 35.28%. The fatty acid composition in the lipid extracts was also analyzed. The methane yield from the residual extracted biomass pretreated by microwaves ranged from 148 to 185 NmL CH₄/g VS for *C. vulgaris* and from 128 to 142 NmL CH₄/g VS for *B. braunii*. In the case of ultrasonic pretreatment, the methane production was between 168 and 208 NmL CH₄/g VS for *C. vulgaris*, while for *B. braunii* ranging from 150 to 174 NmL CH₄/g VS. Anaerobic digestion showed that lipid-extracted biomass presented lower methane yield than non-lipid-extracted feedstock, and higher amount of lipid obtained in the extraction contributed less methane production. Anyway, anaerobic digestion of the residual extracted biomass can be a suitable method to increase economic viability of energy recovery from microalgae.

Keywords Cell disruption · Lipid recovery · Fatty acid methyl esters · Anaerobic digestion · Microalgae

Introduction

Nowadays, there is an increasing demand for energy carriers obtained from renewable sources arising in harmony and respect for the natural environment. Moreover, an important issue is searching for the non-food bioenergy feedstocks to reduce the consumption of the food and feed sources [1]. Due to the rapid growth rate of the microalgae and their easy adaptation to environmental conditions, this biomass is currently considered an alternative feedstock for the production of biofuels replacing the fossil fuels [2, 3]. Microalgae have many intracellular substances that can be widely used in the food and cosmetics industries. They can be also used in the production of the liquid and gaseous biofuels such as biodiesel, bioethanol, biohydrogen, and biogas [4–6]. More

attention has been recently paid to research on lipid extraction from microalgae. According to the literature, microalgae appear to be a promising source for biodiesel production to meet the global demand for transport fuels [7, 8].

Microalgae cells are protected by the complex cell walls which consist of lipid, cellulose, protein, glycoprotein, and polysaccharide. The fundamental cell wall components include a microfibrillar network within a gel-like protein matrix; however, some microalgae are also protected by an inorganic rigid wall composed of silica frustules or calcium carbonate [9]. The crucial step in gaining the bioactive compounds from microalgal biomass is to achieve the efficient cell disruption, which depends on various parameters such as composition of cell wall, location of the desired biomolecule in microalgae cells, and growth stage of microalgae during harvesting [10]. Some species of microalgae, under appropriate conditions, may accumulate within the cells large amounts of lipids (20–70%) [11, 12]. In order to efficiently produce biodiesel from microalgae, strains with a high growth rate and favorable fatty acid methyl ester (FAME) composition have to be selected. The microalgae species that are capable of accumulating much lipid in the cells are *Chlorella* sp., *Botryococcus braunii*, *Porphyridium*, *Nannochlorosis*, *Neochlorosis*, *Dunaliella*,

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and *Scenedesmus* [13, 14]. However, the lipid extraction from algal cells is difficult, because some lipids are bound to the cell membranes. Thus, the microalgae biomass pretreatment prior to direct lipid extraction is necessary to break the cells and violate the cell walls to maximize the lipid recovery [15, 16]. Disintegration of the cellular structure before the lipid extraction has many advantages, such as faster extraction time, less solvent consumption, greater solvent penetration into the cell, and increasing the release of the cell content [15]. Methods for effective microalgal cell disruption include mechanical, physicochemical, and enzymatic techniques. Physical pretreatment was found to be a high-energy and cost-intensive process; however, according to the literature, the most promising method for cell disintegration is the use of microwaves and ultrasounds [17]. Both physical pretreatment methods provoked the cell disruption, but the physical natures of the interaction of ultrasounds and microwaves were not the same. During ultrasonic pretreatment, the energy of high-frequency acoustic waves initiates a cavitation process and a propagating shock wave in the surrounding medium causing cell disruption by high shear forces [18]. The interaction of microwaves involves the conversion of electromagnetic energy into heat as a result of polar particle rotation. The microwaves interact selectively with the dielectric or polar molecules (e.g., water) and cause local heating as a result of frictional forces from inter- and intramolecular movements [19, 20].

The application of ultrasonication to the cell wall disruption is a relatively economical and effective method for biodiesel production from microalgae. Ultrasonic pretreatment is characterized by a lower energy demand than, for example, pressure methods. Ultrasounds generate vibrations that break the cell structure mechanically and improve material transfer by enhancing the extraction of lipids from microalgae. In ultrasonic pretreatment, an important issue is to choose the appropriate operational parameters (i.e., frequency, energy input, pretreatment time), because too intense sonication leads to a significant increase in the temperature, protein denaturation, and liquid foaming [21–23]. The application of ultrasonication is not limited to the extraction of oil from microalgae cells, but may also significantly improve and accelerate a transesterification process [18]. Similar findings were observed by Ranjan et al. [24]. Using the ultrasonic pretreatment before the extraction of *Scenedesmus* sp. biomass by using the Soxhlet method, much higher lipid yield was obtained.

An alternative to ultrasonic pretreatment may be thermal depolymerization with microwave radiation. Molecules with a dipole moment vibrate in the electromagnetic field that leads to temperature increasing and energy forms changing. The advantage of microwave radiation over conventional heating is the formation and propagation of the thermal energy in a relatively short time in the entire volume and mass of the substrate [21, 23]. In a closed system, this causes a significant

increase in a pressure. The heat treatment causes a deep penetration of microwaves through the cell wall structure of microalgae, enhancing the lipid extraction efficiency [25]. The rapid heating leads to a high internal temperature of the treated biomass and a pressure difference affects the cell wall, thus enhancing the mass transfer rate without thermal degradation of lipids [26].

Irrational biomass production and its components may create, depending on the process details, large environmental burdens. That is why it is so important to process biomass in one production cycle by several energy technologies. This will reduce the formation of waste products and emissivity as well as increase the energy yield from biomass. Thus, the technologies of biofuel production should be interrelated in multi-system systems which will enhance the production yield and quality of the products [27]. The use of residual microalgal biomass after the lipid extraction should be considered to increase the profitability of microalgae cultivation. According to the literature, anaerobic digestion can be a suitable method for energy recovery from the lipid-extracted microalgal biomass [28, 29]. The high content of intracellular compounds accumulated in the algal cells causes the microalgal biomass to be a promising feedstock to produce bioenergy [30]. However, many factors may inhibit the anaerobic digestion or significantly reduce the efficiency of biogas production. The literature data have demonstrated the problems related to the availability of intracellular substances for anaerobic microflora due to the cell wall resistance to anaerobic degradation [29]. Thus, the pretreatment step should be used to disrupt the cell wall structure. However, in many cases, there is no economic justification for the pretreatment due to the high costs of feedstock preparation. In this way, the use of initially disintegrated microalgal biomass after the lipid extraction may increase economic viability of the process because the potential of energy gain may be relatively higher than that obtained in unit operations.

Nowadays, microalgae are clearly one of the most promising sources for new-generation biofuels, whereas anaerobic digestion to produce methane is a feasible way to gain bioenergy from microalgae biomass [31]. The methane yield from anaerobic digestion of algae ranging from 140 to 360 mL/g volatile solids (VS) fed the digester, which is comparable to the yield obtained with sewage sludge digestion of 190–430 mL/g VS [32]. Microalgae with a high lipid content are particularly suitable for the production of extract to biodiesel [16]. Based on the principles of circular economy, renewable resources must be used in a sustainable and circular way and residues minimized or completely removed by recycling or re-using. In this way, in our investigations, it was checked as to whether or not the algae residue after the lipid extraction is suitable for fermentation to produce biomethane. In this respect, comparative anaerobic investigations of the raw microalgae biomass (without extraction) and

the residual biomass (after extraction) were carried out, and the methane potential was determined. Additionally, the effects of pretreatment methods (ultrasounds and microwave radiation) on the lipid yield were also assessed.

The first research objective of the study was to compare the efficiency of ultrasonic and microwave pretreatment of *Chlorella vulgaris* and *Botryococcus braunii* microalgae on the lipid yield and composition of the fatty acids in the lipid extracts. The second objective was to determine the efficiency of methane production from the residual microalgal biomass after the lipid recovery.

Materials and Methods

Algae Species and Culture Conditions

Microalgae inoculum of *Chlorella vulgaris* and *Botryococcus braunii* used in the study was originated from the own culture collection (University of Warmia and Mazury in Olsztyn, Department of Environmental Engineering).

The microalgae species were initially cultivated in sterilized reactors with an active volume of 2000 cm³. Pasteurization of the reactors was carried out using a Tuttnauer 2840EL-D autoclave (15 min, 121 °C). The culture medium was the SAG medium dedicated to the particular species of algae, excluding nitrogen compounds [33, 34]. In order to obtain high lipid productivity, a nitrogen regime was used in accordance with the literature [35, 36]. The reactors were incubated in the KBWF climate test chamber with programmable MB1 Binder controller. Cultivation was carried out at 24 °C providing a constant white light (200 mM/m² s). The compressed air (at 50 L/h by a diaphragm pump) was delivered to the reactors to ensure a sufficient mixing of the culture medium and homogeneity of conditions within the entire reactor volume (Table 1). The cultivation time lasted 21 days. After that time, the average biomass concentration of *C. vulgaris* and *B. braunii* was 3800 ± 150 mg volatile solids (VS)/L and 2550 ± 120 mg VS/L, respectively. The tests were carried out in triplicates for each algae species. The biomass concentration was measured by using the weight method. The characteristics of microalgae inoculum are shown in Table 2.

Table 1 Algae cultivation and culture conditions

Algae species	Temperature	Lith exposition	CO ₂ supply, 0.02%	Culture medium	Lipid accumulation
<i>Chlorella vulgaris</i>	24 ± 1 °C	200 μmol m ⁻² s ⁻¹	50 L/h air	SAG synthetic nutrient solution	Nitric cultivation regime
<i>Botryococcus braunii</i>	28 ± 1 °C	200 μmol m ⁻² s ⁻¹	50 L/h air	SAG synthetic nutrient solution with soil extract	Nitric cultivation regime

Table 2 Characteristics of microalgae *C. vulgaris* and *B. braunii*

Parameter	Unit	<i>C. vulgaris</i>	<i>B. braunii</i>
Total solids (TS)	(%)	2.73 ± 0.13	4.01 ± 0.15
VS	(% TS)	93.12 ± 0.97	94.56 ± 1.33
C	(% VS)	58.37 ± 1.75	56.41 ± 2.18
N	(% VS)	2.79 ± 0.43	2.36 ± 0.39
C:N	-	20.9 ± 1.09	23.9 ± 1.28
Protein	(% VS)	27.11 ± 2.72	16.18 ± 2.54
Lipid	(% VS)	26.26 ± 1.64	34.04 ± 3.96
Carbohydrate	(% VS)	39.77 ± 1.29	38.21 ± 2.63

Pretreatment Procedures

Microwave pretreatment (MW) was carried out on the CEM Mars microwave digestion oven (2.45 GHz, 400 W) with four different exposure time (0 s, 10 s, 20 s, 40 s, and 60 s). A total of 50 mL of microalgal biomass was introduced to the reaction chambers. The energy inputs used in the study are shown in Table 3.

In ultrasonic pretreatment (US), the UP400St Hielscher Ultrasonics (Germany) disintegrator with a frequency of 24 kHz, a power of 400 W, and a sonotrode diameter of 10 mm was used. The time of disintegration was as follows: 0 s, 10 s, 20 s, 40 s, and 60 s. The biomass sample volume was 50 mL, and the energy inputs are shown in Table 3. After the disintegration, the pretreated biomass was subjected to the lipid extraction process.

Lipid Extraction and Fatty Acid Profile Analysis

The lipids from microalgae cells were extracted using the chloroform/methanol mixture at a ratio of 2:1 (v/v) according to the modified Bligh and Dyer method [37]. After 48 h of extraction, biomass was centrifuged (6000 rpm, 6 min, Hettich Eba 200) to remove cell debris. Then, the extract was evaporated on a water bath in 60 °C and analyzed by using the weight method.

The determination of FAME was performed by a gas chromatograph (Bruker 450-GC) equipped with a FID detector. A qualitative analysis identified peaks corresponding to different

Table 3 Pretreatment parameters

Variants	Time (s)	The amount of ultrasound input (Ws)	The amount of microwave input (Ws)	<i>C. vulgaris</i> energy doses (Ws/mg VS)	<i>B. braunii</i> energy doses (Ws/mg VS)	Temperature after ultrasound disintegration (°C)	Temperature after microwave disintegration (°C)
Control	0	0	0	0	0	20.0 ± 0.5	20 ± 0.5
1	10	4000	4000	21	31	22.6 ± 0.6	40 ± 2.0
2	20	8000	8000	42	63	28.3 ± 0.6	60 ± 5.0
3	40	16,000	16,000	84	125	36.6 ± 0.9	100 ± 8.0
4	60	24,000	24,000	126	188	44.0 ± 1.7	140 ± 9.0

components of the sample. The identified components were determined by comparing their retention times and fragmentation patterns with standards. The characteristics of FAME in the raw microalgae biomass are shown in Table 4.

Anaerobic Biodegradability Tests

The residual microalgal biomass after the extraction process was evaporated on a water bath to remove residual solvents using lipid extraction. Then, the biomass was directly used as a substrate for anaerobic digestion carried out in Automatic Methane Potential Test System II (AMPTS II) Bioprocess Control (Sweden). The initial organic loading rate (OLR) was established on 5.0 g VS/L (substrate to inoculum ratio was 1:5). The inoculum was collected from a laboratory anaerobic reactor operated with maize silage and cattle manure in mesophilic conditions. Initially, the anaerobic inoculum was fasted for 5 days and then was introduced into the reactor in the volume of 100 mL. The pH of the mixture of inoculum and feedstock was 7.08. A control test was carried out on the biogas productivity of inoculum alone. The results of biogas production are the net values calculated by subtracting the biogas productivity of inoculum alone from the gross value of biogas production. After filling the reaction chambers (500 mL) with the feedstock and inoculum, they were flushed with nitrogen to remove atmospheric air at the beginning of tests

and then incubated at 36 °C and mixed periodically. The produced biogas flowed through the CO₂ absorber and then through the automatic biogas meter. The volume of the produced methane was converted to normal conditions. The total digestion time lasted 40 days.

Analytical Methods

Determinations of VS were carried out by gravimetric analysis. The biomass samples dried at 105 °C were also assayed for contents of total carbon (TC) and total nitrogen (TN) with the use of elementary particle size analyzer (Flash 2000, Thermo Scientific, USA). Carbohydrate content was determined using the YSI enzymatic electrodes (USA). The content of total protein was estimated by multiplying the value of TN by 6.25. The concentration of lipids was assayed by using Soxhlet's method using an extractor (Büchi, Switzerland). The pH of aqueous solutions of anaerobic sludge and algae biomass was determined with a pH meter (1000 L, VWR, Germany).

The composition of biogas was measured using a gas chromatograph (GC, 7890A Agilent) equipped with a thermal conductivity detector (TCD). The GC was fitted with the two Haysep Q columns (80/100 mesh), two molecular sieve columns (60/80 mesh), and Porapak Q column (80/100) operating at a temperature of 70 °C. The temperature of the injection and detector ports was 150 °C and 250 °C, respectively. Helium and argon were used as the carrier gases at a flow of 15 mL/min. The content of methane (CH₄) and carbon dioxide (CO₂) was measured.

Statistical Analysis

The statistical results of the study were analyzed by using the Statistica 10.0 PL package (StatSoft, Inc.) with a Shapiro–Wilk *W* test. One-way analysis of variance (ANOVA) was applied to determine the significance of differences between variables. The significance of differences between the analyzed variables was determined with a Tukey RIR test. In all tests, the level of significance was $\alpha = 0.05$.

Table 4 The characteristics of the fatty acid methyl esters (FAME) in the lipid extracts of microalgae biomass—content in extract (%)

	<i>C. vulgaris</i>	<i>B. braunii</i>
C13:0	12.11	2.46
C16:0	15.88	21.71
C16:1		
C18:0		10.18
C18:1	15.03	33.12
C18:2		
C18:3		8.08
C20:1	46.25	
C20:3	10.72	24.46
C20:4		

Results and Discussion

Lipid Contents of Microalgae Pretreated Biomass and Fatty Acid Composition

Ultrasonic and microwave pretreatment of microalgae biomass was used to enhance the lipid extraction. Additionally, the residual microalgal biomass after the lipid extraction as a feedstock for anaerobic digestion was assessed.

The lipid yield from *B. braunii* biomass without pretreatment was 34.04%, while from *C. vulgaris* was 26.26% ($p < 0.05$) (Fig. 1). Both pretreatment methods significantly enhanced the amount of lipid obtained from both microalgae biomasses. Ultrasonication for 60 s ensured the highest lipid yield from *B. braunii* and *C. vulgaris* of 39.61% and 35.28%, respectively (Fig. 1). Generally, the efficiency of lipid extraction from tested microalgal species after ultrasonication increased with the extended disruption time. In turn, microwave disintegration was more effective in relation to *B. braunii* and the maximum lipid yield was over 56%. This was 16% more comparing with ultrasonication ($p < 0.05$). In the case of *C. vulgaris*, the maximum lipid yield reached 41.31% and was about 6% higher than observed after ultrasonication ($p < 0.05$) (Fig. 1). The highest increase in lipid extraction efficiency was obtained when the disintegration time was extended from 10 to 20 s. However, the pretreatment that lasted for more than 40 s did not cause a significant increase in the amount of extracted lipids (Fig. 1). These results are similar to those obtained by Lee et al. [25]. They tested several

pretreatment methods of *Chlorella* sp. and *Botryococcus* sp. biomass before lipid extraction and noted that the efficiency of microwaving was higher than of ultrasonication. A lipid yield from *Botryococcus* biomass after microwave pretreatment was 28.6%, while the sonication method showed a low efficiency (8.8%). Similarly, higher efficiency of microwave disruption has been also confirmed by McMillan et al. [38] using *Nannochloropsis oculata*. The amount of damaged cells was about 95% for microwaves operating at 74.6 MJ/L, while for ultrasounds (132 MJ/L), it was 67%. Patel et al. [39] proved the effectiveness of ultrasonic and microwave pretreatment for improving lipid extraction from algae biomass. In fatty acid profile, they obtained $10.39 \pm 0.15\%$ of saturated and $76.55 \pm 0.19\%$ of monounsaturated fatty acids qualifying the extracted lipids for the production of high-quality biodiesel.

Both Patel et al. [39] and Ali and Watson [40] stated that the extraction temperature influenced the efficient release of intracellular lipids and the separation of higher unsaturated fatty acids. Moreover, the composition of fatty acids from microalgae biomass differed depending on the algae species and the pretreatment method used.

In this study, the analysis of fatty acids in the lipid extracts of microalgae biomass showed that unsaturated fatty acids constituted the majority in the fatty acid profile (from 55 to 68%) of *B. braunii* extract, and differences between both methods of disintegration were not significant. In the case of *C. vulgaris* biomass, the amount of unsaturated acids in relation to saturated acids increased with pretreatment time extension. Unsaturated acids constituted 25% of the total fatty acids

Fig. 1 Lipid yields (% VS)

in the control sample, 61% after ultrasonic pretreatment, and 75% after microwave disintegration (Fig. 2). The analysis of biodiesel FAME indicated the following major long-chain fatty acids: palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), and γ -linolenic (20:3). In *B. braunii* biomass, significantly higher content of monounsaturated fatty acids in the total fatty acid profile was observed compared with *C. vulgaris* biomass.

According to Martinez-Guerra et al. [21], microwave radiation ensured more efficient lipid recovery from *Chlorella* sp. biomass than ultrasonication. The authors investigated the effects of microwaves and ultrasounds on the extractive-transesterification of lipids from *Chlorella* sp. with using ethanol as a solvent. In microwave-enhanced conditions, polyunsaturated fatty acids (PUFAs) dominated in the extracted lipids (70% on average), while saturated fatty acids (SFAs) constituted over 70% with using ultrasounds. According to them, microwaves selectively extract lipids from the

biological matrix of algae, whereas ultrasounds damage the cell walls and alter the structure of the cells. Moreover, it is possible that ultrasonication enhanced the extraction of undesired substances due to the dominance of disruptive mechanism. On the other hand, microwaves caused a local superheating of the lipid compounds to selectively extract them. However, higher temperatures generated by microwaves may cause the extracted products to oxidize.

Biomethane Potential from the Microalgae Residues

Methane production from pretreated and lipid-extracted microalgae residues of *C. vulgaris* and *B. braunii* was assessed. Biomethane potential tests showed similar methane production from both algal species after the lipid extraction without pretreatment (control) ($p > 0.05$) (Fig. 3). Raw biomass (with lipids) showed the highest methane yield of 250 ± 15 NmL/g VS for *C. vulgaris* and 276 ± 14 NmL/g VS for *B. braunii*. In general, methane productivities were directly

Fig. 2 Comparison of FAME composition in the lipid extracts of microalgae biomass using microwave and ultrasonic pretreatments (SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids)

Fig. 3 Methane production yield

dependent on the amount of extracted lipids. Higher amount of lipid obtained in the extraction contributed less methane production in anaerobic digestion due to the lower content of organic matter in the feedstock (Fig. 3; Fig. 4). An inverse relationship between methane production yield and lipid yield was recognized (Fig. 4). The lipid concentration in the digested biomass mostly influenced the methane yield from ultrasonicated biomass ($R^2 = 0.9084$; Fig. 4). Moreover, the pretreatment method significantly influenced the methane production. The lowest methane yields were observed with biomass pretreated by microwaves (148 NmL/g VS for *C. vulgaris* and 128 NmL/g VS for *B. braunii*). Increasing the ultrasound disruption time above 10 s did not give a significant change in the amount of methane production (Fig. 3). This was related to the amount of lipids recovered. In microwave-pretreated residual biomass, the reduction in methane production occurred along with the extension of

pretreatment time to 40 s. Then, the increase in the microwave dose did not affect the amount of methane produced from *B. braunii* biomass ($p > 0.05$). In the case of *C. vulgaris*, microwave radiation affected the decrease in methane production in all experimental variants.

The methane potential of microalgae biomass is very well recognized [41]. However, a methane yield is strongly dependent on the species of algae used as a biomass feedstock [41]. Zamalloa et al. [42], for instance, reported a methane yield of 210 ± 30.0 L CH_4/kg VS digesting *Scenedesmus obliquus* (Chlorophyta) biomass and 350 ± 30.0 L CH_4/kg VS for *Phaeodactylum tricornutum* (Bacillariophyceae) biomass. On the contrary, according to Mussnug et al. [43], susceptibility of individual algal species and their taxonomic groups to anaerobic digestion is closely related to the structure of their cell walls. The literature results present that the digestibility of six species of phytoplankton commonly occurring in both fresh and salt waters (i.e., *Chlamydomonas reinhardtii*, *Dunaliella salina*, and *S. obliquus* from the class Chlorophyceae; *Chlorella kessleri* from the class Trebouxiophyceae; *Euglena gracilis* from the class Euglenoidea; and blue-green algae *Arthrospira platensis* from the class Cyanophyceae) was not correlated with the algae class [43]. Unpaprom et al. [44] found that biogas production from *B. braunii* was 614.11 L/kg VS with methane concentration of 65.92%.

Thus, in the production of methane in anaerobic digestion, the most important factor is the biomass composition. Methane yield from algae biomass is mostly related to lipid content in the cells [45]. According to Quinn et al. [46], the biomethane potential of the biomass of *Nannochloropsis salina* after the lipid extraction was 140 cm^3 CH_4/g VS, which was three times lower than the raw biomass (430 cm^3 CH_4/g VS). In this study, the difference between methane yield obtained from the raw biomass of *B. braunii* and biomass after the lipid extraction achieved 43%. The lipid-extracted

Fig. 4 Correlation between methane production yield and lipid yield

Table 5 Energy assessment of the residual extracted biomass

	<i>C. vulgaris</i>			<i>B. braunii</i>		
	*Energy value of lipid obtained (kJ)	*Energy value of methane obtained (kJ)	Sum (kJ)	*Energy value of lipid obtained (kJ)	*Energy value of methane obtained (kJ)	Sum (kJ)
Control	4.67	3.47	8.14	6.30	3.38	9.68
USV1	4.94	3.43	8.37	6.50	2.87	9.37
USV2	6.41	2.90	9.31	6.89	2.81	9.69
USV3	6.55	2.87	9.42	7.15	2.79	9.94
USV4	6.53	2.77	9.30	7.33	2.48	9.80
MWV1	5.05	3.05	8.10	6.83	2.34	9.18
MWV2	6.50	2.90	9.40	8.59	2.28	10.87
MWV3	7.15	2.84	9.99	10.05	2.23	12.28
MWV4	7.64	2.44	10.09	10.44	2.11	12.55

*Energy calculation on 0.5 g of organic biomass used in the anaerobic biodegradability test. The energy value of 1 g of algae oil was assumed to be 37 kJ/g. The energy value of methane was 33 kJ/L

microalgal biomass was used in anaerobic co-digestion with rice straw [47]. This allowed for better energy recovery and provided a sustainable approach for the development of a microalgae-based biorefinery for the production of biodiesel and biogas as an energy fuel. Energy assessment of the residual biomass is shown in Table 5. The results show that higher lipid yield resulted in lower methane production. Using residues after the lipid extraction to produce methane significantly improved the energy balance; thus, integrated biomethane and biodiesel production can be a sustainable approach to establish microalgae biorefinery.

Conclusions

In this study, the two physical methods for *Botryococcus braunii* and *Chlorella vulgaris* biomass pretreatment before lipid extraction were examined. Microwave pretreatment provided better results of lipid extraction from *B. braunii* biomass. *C. vulgaris* biomass was less susceptible for these disruption methods. The residual microalgal biomass after the lipid extraction can be a valuable feedstock for biogas production in anaerobic digestion.

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